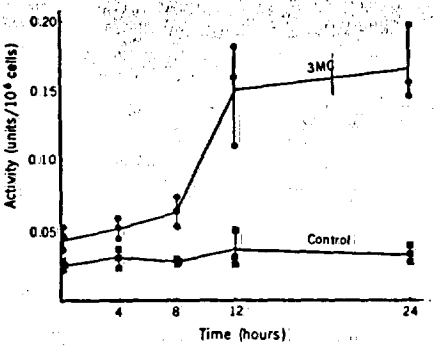
# Aryl Hydrocarbon Hydroxylase Induction in Human Leukocytes

Abstract. *A method for determining aryl hydrocarbon hydroxylase induction in human leukocytes is described. Leukocytes from healthy volunteers were cultured in the presence of phytohemagglutinin, a mitogen. Addition of 3-methylcholanthrene to 72-hour cultures induced a fourfold increase in aryl hydroxylase activity. In the absence of a mitogenic agent, 3-methylcholanthrene stimulation of increased enzymatic activity did not occur.*

Benzo[*a*]pyrene (BP), 3-methylcholanthrene (3MC), and related polycyclic hydrocarbons are carcinogens commonly present in tobacco smoke (*1*), polluted city air (*2*), and certain foods (*3*). The enzyme system aryl hydrocarbon hydroxylase (AHH) functions in the biotransformation of BP and related compounds to hydroxylated metabolites with resultant alteration of their carcinogenic activity. The exact relation between AHH activity and resistance to tumor initiation by environmental carcinogens is not well understood. This inducible enzyme occurs in many mammalian tissues including liver, lung, intestinal mucosa, thyroid, testis, adrenal cortex (*4*), striated muscle (*5*), placenta (*6*), and skin (*7*, *8*). The inducibility of AHH shows genetic variation in the mouse, and is controlled by a single autosomal dominant locus (*9*). If this genetic control is as simple in man the implications would be profound.

Increased activity of AHH has been found in human placenta from cigarette smokers (*6*), and in cultured human foreskin from newborn infants (*8*). The lack of a method for assaying readily available human tissues has confined previous studies of AHH induction and activity to select populations. The demonstration of AHH induction in rat Kupffer cells (*10*) suggested agranular leukocytes (precursors of Kupffer cells) (*11*), may be inducible under appropriate conditions. We describe here a method for measuring the induction of AHH activity in normal human leukocytes.

To 10 ml of heparinized blood we added an equal volume of 3 percent dextran in normal saline and allowed the erythrocytes to sediment for 45 minutes at room temperature. The leukocyte-rich supernatant was centrifuged at 1000*g* for 3 minutes. The pellet was suspended in Gibco chromosome medium 1-A which contains phytohemagglutinin. Culture tubes were prepared containing $2 \times 10^6$ to $4 \times 10^6$ cells in 5 ml of medium. After incubation for specified intervals at 37°C appropriate tubes received 5 $\mu$l of 0.75 m*M* 3MC in methanol. Control tubes received only methanol. The cells were harvested 24 hours after addition of 3MC, and were suspended in 1.0 ml of 50 m*M* tris-HCl, *p*H 7.5, containing 3 m*M* $MgCl_2$ and 0.2*M* sucrose (TMS). Cell counts were made from the 1.0 ml cell suspension after a 1 : 1 dilution of a 25-$\mu$l portion with 1 percent acetic acid containing a trace of gentian violet. Leukocytes were homogenized in a glass tissue grinder, and



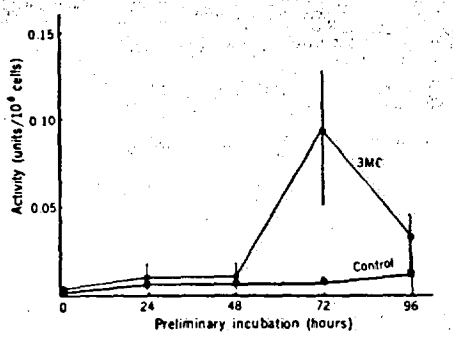




Fig. 1 (left). Time course of AHH induction by 3-methylcholanthrene (*3MC*) Leukocytes were first incubated for 72 hours in medium containing phytohemagglutinin. 3-Methylcholanthrene was added to a final concentration of 0.75 $\mu M$. Cells were harvested at the indicated intervals and were assayed for AHH activity. Each point represents a single determination; vertical bars represent the range of values. A unit of activity is the fluorescence produced equivalent to a picomole of 3-hydroxybenzopyrene per minute. Fig. 2 (right). Response of leukocytes to 3-methylcholanthrene during cultures. Leukocytes were maintained in phytohemagglutinin medium for the indicated times prior to addition of 3-methylcholanthrene and harvested after 24 hours. Each circle represents the mean of six determinations and each square represents the mean of three determinations. The vertical bars give the range of values.